SHORT RESEARCH AND DISCUSSION ARTICLE

Dithiothreitol-based oxidative potential for airborne particulate matter: an estimation of the associated uncertainty

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Abstract

Oxidative stress is considered as one of the main mechanisms by which airborne particles produce adverse health effects. Several methods to estimate the oxidative potential (OP) of particulate matter (PM) have been proposed. Among them, the dithiothreitol (DTT) assay has gained popularity due to its simplicity and overall low implementation cost. Usually, the estimations of OP^{DTT} are based on n-replicates of a set of samples and their associated standard deviation. However, interlaboratory comparisons of OP^{DTT} can be difficult and lead to misinterpretations. This work presents an estimation of the total uncertainty for the OP^{DTT} measurement of PM_{10} and $PM_{2.5}$ samples collected in Santiago (Chile), based on recommendations by the Joint Committee for Guides in Metrology and Eurachem. The expanded uncertainty expressed as a percentage of the mass-normalized OP^{DTT} measurements was 18.0% and 16.3% for PM_{10} and $PM_{2.5}$ samples respectively. The dominating contributor to the total uncertainty was identified (i.e., DTT consumption rate, related to the regression and repeatability of experimental data), while the volumetric operations (i.e., pipettes) were also important. The results showed that, although the OP measured following the DTT assay has been successfully used to estimate the potential health impacts of airborne PM, uncertainty estimations must be considered before interpreting the results.

Keywords Uncertainty estimation · Oxidative potential · DTT assay · PM_{10} · $PM_{2.5}$ · Chile

Introduction

There is enough evidence of the relationship between the exposure to air pollutants such as airborne particulate matter (PM) and negative human health effects (Anderson et al.

The authors, in special MALG, dedicate this work in loving memory of wonderful father Manuel de la Cruz Leiva Angulo (1927–2019). "Death is a stripping away of all that is not you. The secret of life is to die before you die and find that there is no death" (Eckhart Tolle, in "The Power of Now").

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2012; Gouveia et al. 2018; Kim et al. 2015). Oxidative stress has been considered as one of the main mechanisms by which PM can trigger negative health effects, due to the generation and subsequent reactions of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Araujo 2011; Cheng et al. 2012; Donaldson et al. 2001; Molina et al. 2020; Schins et al. 2004; Tuet et al. 2016). When the ROS/RNS exceed the cellular antioxidant defenses, they can lead to inflammatory processes and apoptosis or cellular carcinogenesis (Borm et al. 2007; Ghio et al. 2012). The production of ROS/RNS can be catalyzed by certain PM constituents with relatively high redox activity (e.g., polycyclic aromatic compounds, quinones, transition metals) (Øvrevik et al. 2015; Piacentini et al. 2019). PM oxidative potential (OP) can thus be defined as a measure of the ability of its components (and physical properties) to oxidize a target molecule or to catalyze the production of ROS/RNS and consumption of antioxidants (Borm et al. 2007; Sauvain et al. 2013; Calas et al. 2019).

The dithiothreitol assay (DTT) is one of several methods that have been proposed to measure the OP of PM (OP^{DTT}) (Ayres et al. 2008; Bates et al. 2019; Hedayat et al. 2015; Fang

et al. 2016; Valavanidis et al. 2005; Pal et al. 2014; Delaval et al. 2017). It has been widely used in recent years, due to its simplicity and relatively low implementation cost (Cho et al. 2005; Li et al. 2009). It is based on a UV-Vis spectrophotometric measurement of the consumption rate of DTT in a PM aqueous extract (Kumagai et al. 2002). DTT can act as a surrogate of the biological reducing agent nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) and interacts with some PM components to produce superoxide radicals (Shirmohammadi et al. 2017; Kumagai et al. 2002; Koehler et al. 2014). Several studies have used this method to quantify and compare the OP generated by the different sizes of PM collected from different sources (Charrier et al. 2015; Cheung et al. 2010; Holmen et al. 2017; McWhinney et al. 2013). Results are generally reported as the arithmetic average of a set of replicates with its associated standard deviation (SD). However, interlaboratory comparisons have shown to be difficult due to the lack of standardized protocols including the type of filters used to collect atmospheric PM, solvent and extraction methods used, initial DTT concentrations, incubation time, among others (Jiang et al. 2019; Wampfler and Rösslein 2009; Jedynska et al. 2017; Bates et al. 2019; Lin and Yu 2019). Additionally, the presence of Cu and Mn in high relative concentrations has shown to affect the kinetics of the DTT assay, further complicating a direct comparison between samples from different origins (Charrier et al. 2015; Charrier et al. 2016; Lin and Yu 2019). Therefore, many researchers have concluded that a standardization of the methodology and a better estimation of the total uncertainty associated with the process are necessary (Wampfler and Rösslein 2009; Eurachem 2000; Valcárcel and Ríos 1998; Riu et al. 1999).

The uncertainty is a parameter describing the statistical dispersion of the values attributed to a measured quantity (JCGM100:2008 2008). It permits to establish a level of confidence regarding the quality of the measurement and if it is suitable for its intended purpose (Leiva et al. 2013; Taverniers et al. 2004). The uncertainty associated with the repeatability and linear regression model used in the DTT assay has been estimated before (Weber et al. 2018; Berg et al. 2020). However, a step-by-step description of the uncertainty of the full experimental process has not been reported. This work aims to estimate the total uncertainty associated with the OP^{DTT} measured in atmospheric PM$_{10}$ and PM$_{2.5}$ samples collected in Santiago (Chile). We identified the main contributors to the total uncertainty and established a standardized protocol for the OP^{DTT} estimation. This could help researchers to reduce or replace steps with high uncertainty contributions, increasing the reliability and overall quality of data derived from OP^{DTT}. However, further evaluation will be needed if a different DTT estimation protocol is used.

Materials and methods

Reagents and standards

DL-Dithiothreitol (DTT, ≥ 99.5%, CAS: 3483-12-3), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB, ≥ 98%, CAS: 69-78-3), Chelex® 100 sodium form (CAS: 11139-85-8), 9,10-phenanthrenequinone (PQN, ≥ 99%, CAS: 84-11-7), potassium phosphate dibasic (K$_2$HPO$_4$, ≥ 98%, CAS: 7758-11-4), and potassium phosphate monobasic (KH$_2$PO$_4$, ≥ 98.0%, CAS: 7778-77-0) were purchased from Sigma-Aldrich (St. Louis, MO). Dimethyl sulfoxide (DMSO, CAS: 67-68-5) and methanol (CAS: 67-56-1) were purchased from Merck (Darmstadt, Germany).

PM sampling

Atmospheric PM$_{10}$ and PM$_{2.5}$ samples were collected between June and August 2018 in Santiago (Chile) ($n = 60$) using two high-volume samplers from MCV, S.A. (Barcelona, Spain; model CAV-A /Mb) with size-selective inlets and glass-fiber filters (GF/A, 150 mm in diameter) from Whatman (Maidstone, UK) at a nominal flow rate of 30 m^3 h^{-1} for 24 h. GF/A filters were weighed pre- and post-deployment under controlled conditions (i.e., 20 °C, 50% RH) and using an analytical balance (± 0.01 mg, Radwag XA 110/4Y, Radwag, Torunska, Poland) to determine the total mass of collected PM$_{10}$ and PM$_{2.5}$. Collected samples were covered with aluminum foil to protect them from direct light and stored at −20 °C until analysis.

DTT assay

A full description of the DTT assay can be found elsewhere (Cho et al. 2005; Li et al. 2009). Here, we use a modified version of it, by fixing the initial DTT concentration at 100 μM, setting the incubation temperature at 37 °C and by not using trichloroacetic acid or a chelating agent (Lin and Yu 2019). Briefly, three subsections (1-in. diameter) of each impacted filter were extracted by sonication (30 min) in 45 mL of ultrapure water (18 Ohm). The resulting extracts were filtered using a 0.45-μm PTFE syringe filter (Simsii, Port Irvine, CA). 3.5 mL aliquot of the filtered extract was transferred to a 15-mL acid-washed vial, and 0.5 mL of a DTT stock solution (1 mM) and 1.0 mL of phosphate buffer (pH 7.4, KBP, K$_2$HPO$_4$: KH$_2$PO$_4$, 1:5) were added. The resulting mixture was incubated at 37 °C (Boekel model 113,004, Boekel Scientific, Feasterville, PA) and shaken up using an orbital shaker (model KJ201BD, Chang Bioscience Inc., Freemont, CA). Then, 0.5 mL of aliquots was taken from the reaction mixture every 5 or 10 min and transferred to another vial and 1 mL of a DTNB solution (1 mM) was then added to each aliquot and mixed in a vortex (MX-S model, Scilogex Rocky

Hill, CT), and the product 2-nitro-5-thiobenzoic acid (TNB) was quantified using a UV-Visible spectrophotometer (Agilent/HP 8453, Agilent Technology, Santa Clara, CA) in a 1400-μL quartz cell (Thorlabs Inc., Newton, NJ) at 412 nm (molar absorptivity coefficient of 14,150 M^{-1} cm^{-1}). The DTT consumption rate was estimated using the slope of the curve with at least 5 measured points from $t = 0$ min to $t = 30$ min. Since DTT, DTNB, and TNB are sensitive to light, the analysis was performed with the lights off, in amber glassware, and by covering vials and flasks with aluminum foil (Charrier and Anastasio 2012).

Laboratory blanks (ultrapure water, BLK, $n = 15$), filter blanks (BLF, $n = 18$), and positive controls (PQN, stock solution in dimethyl sulfoxide, 0.21 nmol mL^{-1} in the reaction vial, $n = 15$) were used for QA/QC purposes (Fig. S1). A complete description of the analytical process is shown in Fig. S2.

Estimation of the uncertainty

The total uncertainty of the method was estimated using the "Guide to the Expression of Uncertainty in Measurement" developed by the Joint Committee for Guides in Metrology (JCGM100:2008 2008) and "Quantifying Uncertainty in Analytical Measurement" developed by Eurachem (Eurachem 2000). The process was divided into four steps: description of the measurement procedure (already well defined in the literature), the definition of the relationship between the measurand and the variables, identification, and quantification of sources of uncertainty, and estimation of combined and expanded uncertainties. A nested experimental design was used to estimate the uncertainty on selected PM_{10} ($n = 1$), $PM_{2.5}$ ($n = 1$), and field blank samples ($n = 1$). Six aqueous extracts ($j = 1, 2..., 6$) for each sample selected were measured in triplicate ($i = 1, 2, 3$) (Fig. S3).

Measurement model

The relationship between the measurand (OP^{DTT}) and the variables is summarized in Eq. (1):

$$OP^{DTT}_{m \text{ or } V} = \sigma^{DTT}_{PM} \left(\frac{V_m V_e}{V_s} \right) \left(\frac{A_f}{A_P} \right) \left(\frac{1}{m_{PM} \text{ or } V_a} \right) f_R \, 10^3 \quad (1)$$

where $OP^{DTT}_{m \text{ or } V}$ is the OP normalized per PM mass or volume of air originally sampled in units of pmol DTT min^{-1} μg^{-1} or pmol DTT min^{-1} m^{-3}, respectively; σ^{DTT}_{PM} is the consumption rate of DTT (blank corrected by using the average of field blanks) in units of μM DTT min^{-1}; V_m, V_s, and V_e are the volumes (in mL) of the reaction mixture, the aliquot taken from the aqueous extract, and the total extraction volume, respectively; A_f and A_P are the total area of the impacted filter and the subsection of

the filter used for the aqueous extraction in cm^2; m_{PM} is the mass of PM in the filter expressed as μg; V_a is the total volume of air sampled in 24 h in m^3; f_R is the reproducibility factor for the analysis of the n replicate of a positive control (unitless) and 10^3 is a transformation factor.

Identification and quantification of the sources of uncertainty

The main sources of uncertainty for each section of Eq. (1) were described in a cause-effect diagram (Fig. 1). This included uncertainties associated with the sample collection, DTT consumption rate, linear regressions, a reproducibility factor, and volumetric, area, and mass measurements. Equations (2) to (6) were used to estimate the individual uncertainties related to each source. Further details can be found in the Supplementary Information (SI) file.

$$u_{\sigma_y} = \left(u^2_{\sigma_{y-L}} + u^2_{\sigma_{y-R}} \right)^{1/2} \quad (2)$$

$$u_{A_{f \text{ or } p}} = \left(\left(\frac{\partial A_{f \text{ or } p}}{\partial r_{f \text{ or } p}} \right)^2 u^2_{r_{f \text{ or } p}} \right)^{1/2} \quad (3)$$

$$u_{m_{PM}} = \left(u^2_{m_{w2}} + u^2_{m_{w1}} \right)^{1/2} \quad (4)$$

$$u_{V_a} = \left(\begin{array}{c} \left(\frac{\partial V_a}{\partial F} \right)^2 u^2_F + \left(\frac{\partial V_a}{\partial T_a} \right)^2 u^2_{T_a} + \\ \left(\frac{\partial V_a}{\partial P_a} \right)^2 u^2_{P_a} + \left(\frac{\partial V_a}{\partial t_s} \right)^2 u^2_{t_s} \end{array} \right)^{1/2} \quad (5)$$

$$u_{f_R} = f_R \left(\left(\frac{u_{OP^{obs}_{PQN}}}{OP^{obs}_{PQN}} \right)^2 + \left(\frac{u_{OP^{ref}_{PQN}}}{OP^{ref}_{PQN}} \right)^2 \right)^{1/2} \quad (6)$$

Equation (2) represents the uncertainty associated with DTT consumption rate for a PM aqueous extract or filter blank (u_{σ_y}). It was estimated by combining the uncertainty of the slope obtained from the linear regression ($u_{\sigma_{y-L}}$) and by considering the repeatability of the analysis ($u_{\sigma_{y-R}}$). Eq. (3) represents the uncertainty associated with the area of the collecting filter or the section of it used for the extraction ($u_{A_{f \text{ or } p}}$) and it was estimated based on the uncertainty associated with their measured radii ($u_{r_{f \text{ or } p}}$). The uncertainty of the measured PM mass ($u_{m_{PM}}$) was obtained by combining the uncertainties of the impacted filter ($u_{m_{w2}}$) and non-impacted filter ($u_{m_{w1}}$) (Eq. (4)). Similarly, Eq. (5) represents the uncertainty associated with the sampling volume (u_{V_a}) and was estimated based on the uncertainties associated with the sampling flow rate (u_F), ambient temperature (u_{T_a}), and pressure (u_{P_a}), and
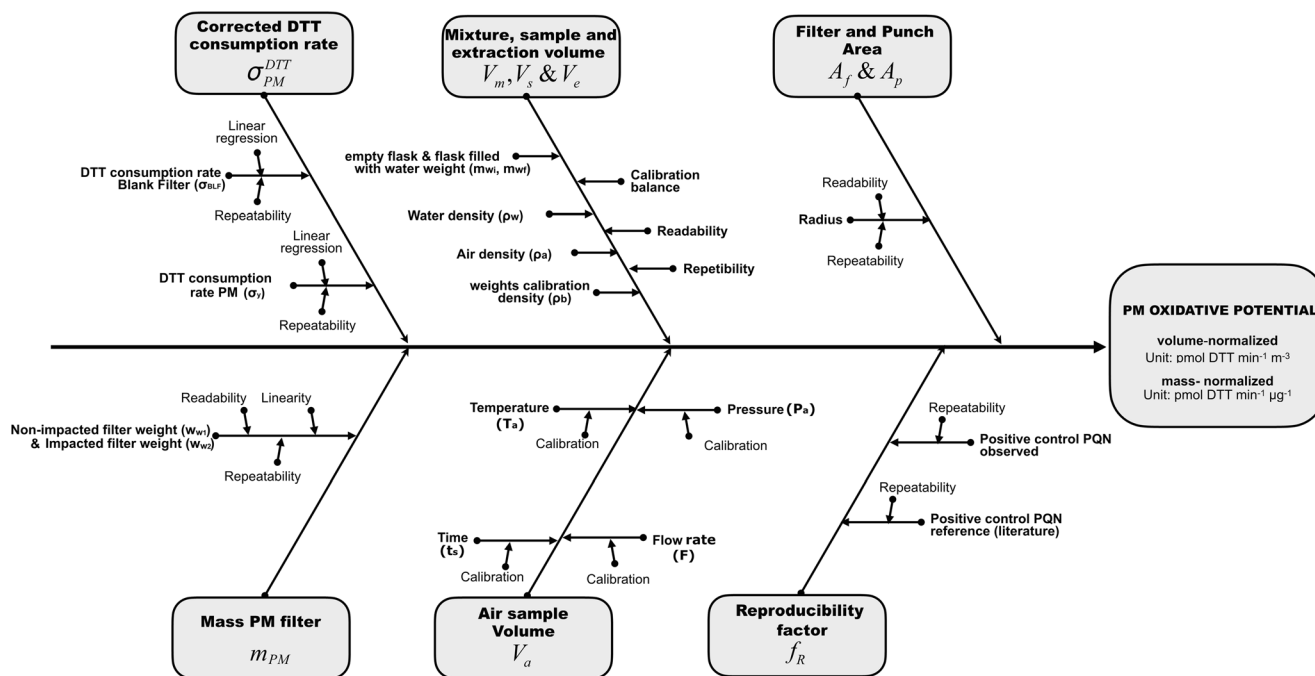
Fig. 1 Cause-effect diagram for OP^{DTT} estimation

the total sampling time (u_{t_s}). Finally, Eq. (6) express uncertainty for reproducibility (u_{f_R}), where $u_{OP_{PQN}^{obs}}$ and $u_{OP_{PQN}^{ref}}$ are uncertainties related to repeatability for the positive control for this study and a reference value.

Estimation of combined and expanded uncertainties

The combined uncertainty ($u_{OP_{m \, or \, V}^{DTT}}$) can be estimated by replacing (u_{q_i}) in Eq. (7) with the individual uncertainties of each term from eq. (1):

$$u_{OP_{m \, or \, V}^{DTT}} = \left(\sum \left(\frac{\partial OP_{m \, or \, V}^{DTT}}{\partial q_i} \right)^2 u_{q_i}^2 \right)^{1/2} \qquad (7)$$

The expanded uncertainty ($U_{OP_{m \, or \, V}^{DTT}}$), defined as an interval within which the value of the measurand is believed to lie with a high level of confidence (EURACHEM 2000), can be estimated by using a coverage factor (k) at a specified confidence level (95% in this study) following Eq. (8):

$$U_{OP_{m \, or \, V}^{DTT}} = \kappa \, u_{OP_{m \, or \, V}^{DTT}} \qquad (8)$$

Finally, the OP results can be expressed using the expanded uncertainty as shown in Eqs. (9) and (10):

$$OP_m^{DTT} \pm U_{OP_m^{DTT}} \; (\mu M \; DTT \; min^{-1} \mu g^{-1}) \qquad (9)$$

$$OP_V^{DTT} \pm U_{OP_V^{DTT}} \; (\mu M \; DTT \; min^{-1} m^{-3}) \qquad (10)$$

Results and discussion

Particulate matter concentration and method validation

The average PM concentrations (± RSD: relative standard deviation) during the sampling period were 47.5 (± 39%) μg m^{-3} (PM$_{2.5}$) and 83.2 (± 38%) μg m^{-3} (PM$_{10}$). One sample for each PM size with concentrations close to the average of all samples collected (85 μg m^{-3} for PM$_{10}$ and 50 μg m^{-3} for PM$_{2.5}$) was selected for the estimation of the uncertainty associated with the DTT assay. Limits of detection (LOD = 0.09 μM DTT min^{-1}) and quantitation (LOQ = 0.30 μM DTT min^{-1}) were determined using the SD of the BLF analyzed (3 and 10 times the SD of the 18 replicates, respectively) (Shrivastava and Gupta 2011). Positive controls (PQN, n = 15) showed an average DTT consumption rate (± SD) of 1.105 (± 0.10) μM DTT min^{-1} (Table 1).

Identification and quantification of uncertainty

A full mathematical description of the sources of uncertainty, including the equations derived from the cause-effect diagram and the results obtained for each source and their contributors, is available in the SI (Table S1).

DTT consumption rate uncertainty

The average DTT consumption rate for BLF was −0.32 μM DTT min^{-1} and for samples analyzed (not corrected for blanks) were − 0.96 (PM$_{2.5}$) and − 1.20 μM DTT min^{-1}

Table 1 Summary statistics of DTT consumption for QA/QC, PQN and PM samples

(a) QA/QC	BLF	BLK	PQN
Time frame (min)	0–30	0–30	0–30
Measured points per extract	5	5	5
Number of replicates	18	15	15
Average slope of the curve (μM DTT min^{-1})	− 0.316	− 0.313	− 1.105
SD of the slope (μM DTT min^{-1})	0.03	0.03	0.10
Average correlation coefficient	0.96	0.96	0.95
Combined uncertainty (μM DTT min^{-1})	0.03	–	–
(b) PM samples	BLF	PM$_{2.5}$	PM$_{10}$
Number of replicates	–	18	18
Average concentration (μg m^{-3})	–	50.0	85.0
Measured points per sample (DTT assay)	–	5	5
Average slope (μM DTT min^{-1})	–	− 0.96	− 1.20
SD of the slope (μM DTT min^{-1})	–	0.05	0.06
Linear regression uncertainty (μM DTT min^{-1})	0.03	0.04	0.07
Uncertainty of repeatability (μM DTT min^{-1})	0.01	0.01	0.01
Combined uncertainty (μM DTT min^{-1})	0.03	0.05	0.08
(c) PQN (reproducibility study)	OP_{PQN}^{ref}	OP_{PQN}^{obs}	
Average of the slope of the curve (μM DTT min^{-1})	− 0.74	− 0.73	
Number of replicates	5	5	
SD (μM DTT min^{-1})	0.01	0.06	
Uncertainty (μM DTT min^{-1})	0.01	0.02	
Reproducibility factor	0.98[a]		
Uncertainty of reproducibility	0.02		
Critical value ($p = 0.01$)	0.95		
2-tailed critical value	2.8		

[a] 0.98 corresponds to the real reproducibility factor estimated by Eq. S20

(PM$_{10}$) (Table 1, section b, line 4). No significant differences were observed between replicates of the same samples (two-factor ANOVA, $p > 0.05$). The combined uncertainty for DTT consumption rate was estimated from Eq. (2), which considers the uncertainty of repeatability and linear regression for filter blanks (σ_{BLF}) and aqueous extracts of PM ($\sigma_{PM_{10}}$ and $\sigma_{PM_{2.5}}$). Results showed that combined uncertainties (with percent relative uncertainty in parenthesis) for DTT consumption rate were 0.03 (9.0%), 0.07 (5.8%), and 0.04 (4.2%) μM DTT min^{-1} for σ_{BLF}, $\sigma_{PM_{10}}$, and $\sigma_{PM_{2.5}}$, respectively (Table 1, section b, line 8).

Reproducibility study

The reproducibility of the DTT assay was estimated by comparing the DTT consumption rate of a standard solution of PQN measured in this study (OP_{PQN}^{obs}) with other previous reports in the literature (OP_{PQN}^{ref}) (Fang et al. 2015). The observed and reference values were not statistically different (t-est., $p = 0.01$), and therefore, the reproducibility factor (f_R)

(Eq. S20 in the SI) was assumed to be 1 (calculated as 0.98) (Table 1). Other studies have also shown similar DTT consumption rates for PQN standards (Gao et al. 2017; Wang et al. 2019). However, we chose to compare our results with one sharing a similar initial DTT concentration and reporting a SD and number of replicates associated with their analysis of a PQN standard solution. Additionally, 1,4-naphthoquinone (1,4-NQ) has also been used as an external standard and could also be used to evaluate the reproducibility among studies (Li et al. 2009; Li et al. 2019).

Combined and expanded uncertainty

OP^{DTT} rates normalized per mass and volume (± RSD) for analyzed samples were 8.9 (± 6%) pmol DTT min^{-1} μg^{-1} and 756 (± 5%) pmol DTT min^{-1} m^{-3} (PM$_{10}$) and 11.1 (± 4%) pmol DTT min^{-1} μg^{-1} and 553 (± 5%) pmol DTT min^{-1} m^{-3} (PM$_{2.5}$) (Table 2). Associated combined and expanded uncertainties were estimated by using Eqs. (7) and (8), with a coverage factor (k) equal to 2 and a 95% confidence level. The relative expanded uncertainty for $OP_{m\ or\ V}^{DTT}$ was

Table 2 Combined ($u_{OP_{m\ or\ V}^{DTT}}$) and expanded ($U_{OP_{m\ or\ V}^{DTT}}$) uncertainty for oxidative potential normalized per mass and volume ($OP_{m\ or\ V}^{DTT}$) for PM_{10} and $PM_{2.5}$ samples

$OP_{m\ or\ V}^{DTT}$	OP^{DTT}	RSD (%)	$u_{OP_{m\ or\ V}^{DTT}}$	$U_{OP_{m\ or\ V}^{DTT}}$	Units	$U_{OP_{m\ or\ V}^{DTT}}$ (%)
$OP_{m\ PM_{10}}^{DTT}$	8.90	5.3	0.8	1.6	pmol DTTmin^{-1} μg^{-1}	18.0
$OP_{m\ PM_{2.5}}^{DTT}$	11.1	4.7	0.9	1.8		16.3
$OP_{V\ PM_{10}}^{DTT}$	756	5.3	70	140	pmol DTTmin^{-1} m^{-3}	18.5
$OP_{V\ PM_{2.5}}^{DTT}$	553	4.7	45	90		16.3

18.0% and 16.3% for PM_{10} and $PM_{2.5}$ samples (Table 2). These results represent the maximum dispersion that the OP^{DTT} value would have if the analysis is performed under the described conditions. In this context, it is expected that standard deviation values will fall within the range of the expanded uncertainty reported. If not, this could indicate the presence of uncontrolled sources of uncertainty in the process.

The main contributors to the total uncertainty of DTT assay were identified: DTT consumption rate, the reproducibility factor, the extraction and reaction mixture volume (i.e., volumetric operations), and air sampling volume (i.e., sampling flow) (Fig. 2). This revealed the importance of establishing standardized protocols for filter, laboratory, and field blanks. The implementation of regular quality control and quality assurance procedures is recommended, particularly for gravimetric and volumetric instruments. This study used pipettes for volumetric measurements. However, the use of an automatic dispenser would represent a 2 to 3% reduction in the estimated uncertainty (this value was estimated by replacing the uncertainty of extraction volume related to pipette operations with the uncertainty given by the automatic dispenser manufacturer in Eq. (7)). On the other hand, the uncertainty
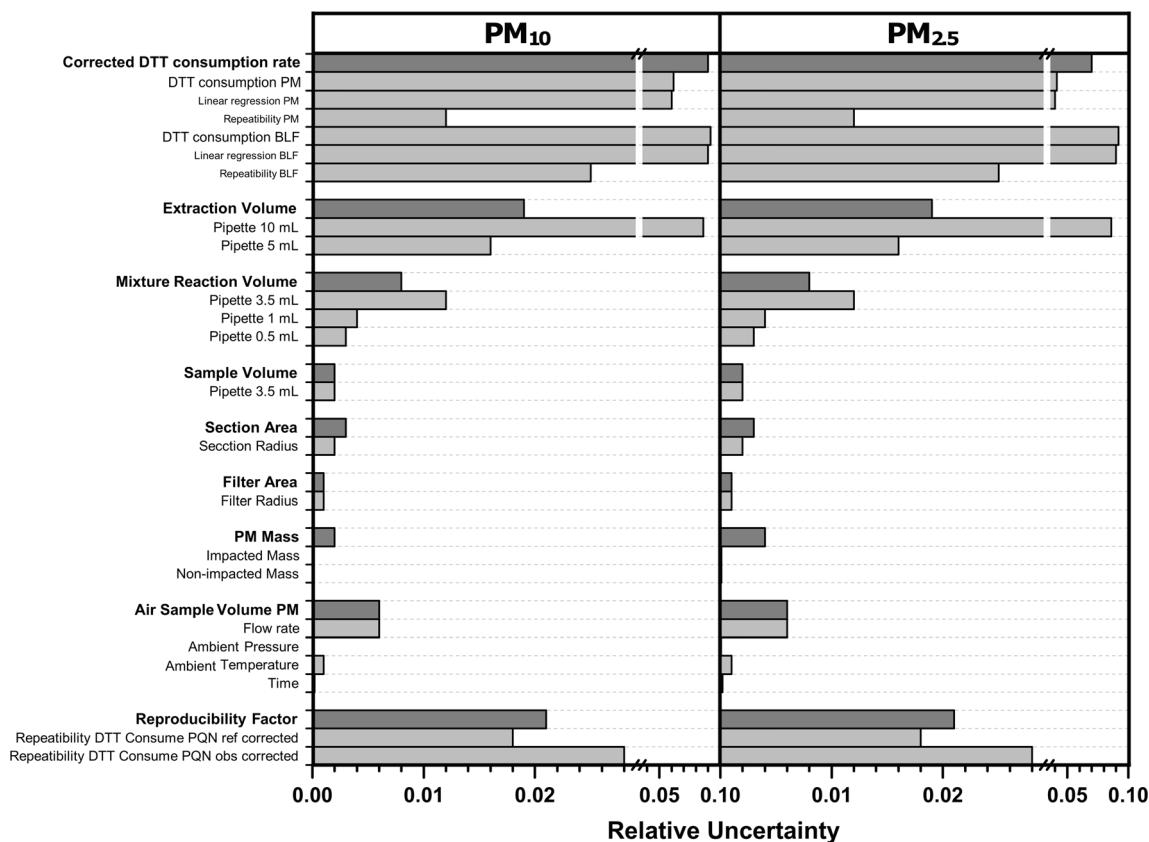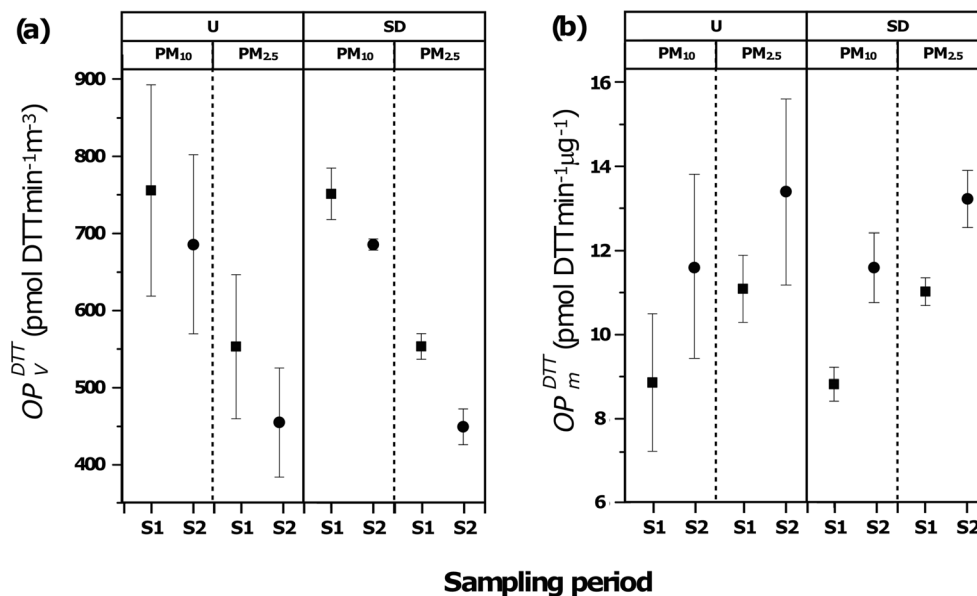

Fig. 2 Relative uncertainties for each identified source of uncertainty

Fig. 3 Expanded uncertainty vs standard deviation of OP^{DTT} normalized for PM_{10} and $PM_{2.5}$ per mass and volume for samples S1 and S2

associated with the use of the sampling equipment was estimated to be 7%, which should be similar for other protocols using the same sampling method. This could be a good starting point for future studies to evaluate the uncertainty of other DTT assay protocols to help standardized the method with the lowest overall uncertainty. One of the limitations of this study is that the chemical composition of the samples collected was not evaluated prior to the estimation of the uncertainty. Therefore, we cannot infer how the different compositions affect the kinetics of the reactions involved. Additionally, the repeatability factor could have overestimated some other uncertainty parameters in our calculations, since several experimental uncertainties are included in it. However, we believe it should be considered because it also includes random errors coming from the operator.

Standard deviation versus uncertainty

Data obtained from DTT assay are commonly expressed as the average of n-replicates and their respective SD. This way of reporting results can lead to erroneous conclusions by not considering the sources of uncertainty of measurements. For example, the OP_m^{DTT} and OP_V^{DTT} were estimated for another set of PM_{10} and $PM_{2.5}$ samples collected at the same site and during the same period (analyzed in triplicate) (Fig. 3). When comparing results and considering only one SD, it could be concluded that the OP^{DTT} is different between sampling periods S1 and S2 for both PM_{10} and $PM_{2.5}$ (Fig. 3). However, if the uncertainty is considered, the range of possible values within which the true value of each measurement lies is broader and may overlap between sampling periods studied. This highlighted the importance of considering the uncertainty in the analysis of environmental samples in order

to draw appropriate conclusions at different spatial and/or temporal scales. An adequate estimation of uncertainty, controlled conditions, and well-understood measurement procedures can help to improve the quality and comparability of measurements, avoiding inappropriate interpretations of results.

Supporting information

Further information regarding the derived equations used to calculate the total and expanded uncertainty are shown in the supporting information file. This file also includes uncertainty values for each step of the experimental process, a flowchart of the method used, a description of the nested experimental design, and a list of all abbreviations used in this manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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